Amendments to the Specification

Please replace the paragraph beginning on page 3, line 7, with the following amended paragraph:

~~PCTs US98/21193~~ WO99/18434, ~~PCT US99/14387~~ WO99/67641 and ~~PCT US98/05025~~ WO 98/40726; WO98/50782, [[and]] U.S.S.N.s 09/287,573, ~~09/151,877, 09/256,943~~, 09/316,154 (US 20020051971), 60/119,323, ~~09/315,584~~; and U.S.P.N.s 6,327,410, 6,429,027, and 6,544,732; all of which are expressly incorporated by reference, describe novel compositions utilizing substrates with microsphere arrays, which allow for novel detection methods of nucleic acid hybridization.

Please replace the paragraph beginning on page 4, line 25, with the following amended paragraph:

The present invention capitalizes on previous work directed to the use of arrays of microspheres or beads that carry different chemical functionalities (such as proteins and nucleic acids) and are distributed on a substrate comprising a patterned surface of discrete sites that can associate the individual microspheres. In these arrays, the beads are generally put onto the substrate randomly and thus require the use of encoding/decoding systems that can correlate the location of the bead with the chemical functionality. This can be done in a variety of ways; see for example ~~PCTs US98/21193~~ WO99/18434; and U.S.S.N.s 09/473,904; 60/119,323; ~~09/315,584;~~ 60/160,027; 60/161,148; 60/135,053; 09/425,633; 60/160,917; 09/287,573; ~~09/151,877; 09/450,829;~~ and 60/151,668; and U.S.P.N.s 6,544,732; 6,327,410; 6,266,459.

Please replace the paragraph beginning on page 9, line 12, with the following amended paragraph:

The present invention provides array compositions comprising substrates with surfaces comprising discrete sites. By "array" or "biochip" herein is meant a plurality of target analyte sets in an array format; the size of the array will depend on the composition and end use of the array. That is, each site on the array comprises a set of target analytes. Nucleic acids arrays are known in the art, and can be classified in a number of ways; both ordered arrays (e.g. the ability to resolve chemistries at discrete sites), and random arrays are included. Ordered arrays include, but are not limited to, those made using photolithography techniques (Affymetrix

GeneChip™), spotting techniques (Synteni and others), printing techniques (Hewlett Packard and Rosetta), three dimensional "gel pad" arrays, etc. A preferred embodiment utilizes microspheres on a variety of substrates including fiber optic bundles, as are outlined in ~~PCTs US98/21193, PCT US99/14387 and PCT US98/05025;~~ WO99/18434, WO99/67641, WO98/40726,WO98/50782; ~~and U.S.S.N.s 09/287,573, 09/151,877, 09/256,943, 09/316,154, 60/119,323, 09/315,584;~~ U.S.S.N.s 09/287,573, 09/316,154 (US 20020051971), 60/119,323; and U.S.P.N.s 6,327,410, 6,429,027, and 6,544,732, all of which are expressly incorporated by reference. While much of the discussion below is directed to the use of microsphere arrays on substrates such as fiber optic bundles, any array format of nucleic acids on solid supports may be utilized.

Please replace the paragraph beginning on page 11, line 1, with the following amended paragraph:

In a preferred embodiment, the substrate is an optical fiber bundle or array, as is generally described in U.S.S.N.s 08/944,850, 09/287, 573 and ~~08/519,062~~ US Patent No. 6,200,737; ~~PCT US98/05025~~ WO98/40726, and PCT US98/09163, and WO 99/18434, all of which are expressly incorporated herein by reference. Preferred embodiments utilize preformed unitary fiber optic arrays. By "preformed unitary fiber optic array" herein is meant an array of discrete individual fiber optic strands that are co-axially disposed and joined along their lengths. The fiber strands are generally individually clad. However, one thing that distinguished a preformed unitary array from other fiber optic formats is that the fibers are not individually physically manipulatable; that is, one strand generally cannot be physically separated at any point along its length from another fiber strand.

Please replace the paragraph beginning on page 13, line 3, with the following amended paragraph:

In a preferred embodiment, physical alterations are made in a surface of the substrate to produce the sites. In a preferred embodiment, the substrate is a fiber optic bundle and the surface of the substrate is a terminal end of the fiber bundle, as is generally described in ~~08/818,199 and 09/151,877~~ U.S. Patent Nos. 6,023,540 and 6,327,410, both of which are hereby expressly incorporated by reference. In this embodiment, wells are made in a terminal or distal end of a fiber optic bundle comprising individual fibers. In this embodiment, the cores of the

individual fibers are etched, with respect to the cladding, such that small wells or depressions are formed at one end of the fibers. The required depth of the wells will depend on the size of the beads to be added to the wells.

Please replace the paragraph beginning on page 17, line 8, with the following amended paragraph:

This pool is then added to a bead; thus, the set of target sequences is known for a bead subpopulation. In addition, although not discussed specifically herein, there are a wide variety of known methods for amplification of nucleic acids. Briefly, these techniques can be classified as either target amplification or signal amplification. Target amplification involves the amplification (i.e. replication) of the target sequence to be detected, resulting in a significant increase in the number of target molecules. Target amplification strategies include the polymerase chain reaction (PCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Alternatively, rather than amplify the target, alternate techniques use the target as a template to replicate a signalling probe, allowing a small number of target molecules to result in a large number of signalling probes, that then can be used as the target analyte. Signal amplification strategies include the ligase chain reaction (LCR), cycling probe technology (CPT), invasive cleavage techniques such as Invader™ technology, Q-Beta replicase (QβR) technology. See U.S.S.N. 60/161,148, ~~09/517,945,~~ 09/553, 993 and 090/556, 463, and U.S. Patent No. 6,355,431; hereby expressly incorporated by reference in their entirety.

Please replace the paragraph beginning on page 18, line 1, with the following amended paragraph:

Thus, in a preferred embodiment, the microspheres of the invention comprise optical signatures or optical codes. That is, as outlined in ~~U.S. patent 6,023,540 and U.S.S.N.s 09/151,877 and 09/450,829~~U.S. Patent Nos. 6,023,540, 6,327,410 and 6,266,459 (all of which are expressly incorporated herein by reference), each subpopulation of microspheres can comprise a unique optical signature or optical tag that is used to identify the target analyte set of that subpopulation of microspheres; that is, decoding utilizes optical properties of the beads such that a bead comprising the unique optical signature may be distinguished from beads at other locations with different optical signatures. Thus the previous work assigned each capture probe a unique optical signature such that any microspheres comprising that capture probe are

identifiable on the basis of the signature. These optical signatures comprised dyes, usually chromophores or fluorophores, that were entrapped or attached to the beads themselves. Diversity of optical signatures utilized different fluorochromes, different ratios of mixtures of fluorochromes, and different concentrations (intensities) of fluorochromes.

Please replace the paragraph beginning on page 21, line 18, with the following amended paragraph:

In addition, the use of different concentrations or densities of IBLs allows a "reuse" of sorts. If, for example, the bead comprising a first agent has a 1X concentration of IBL, and a second bead comprising a second agent has a 10X concentration of IBL, using saturating concentrations of the corresponding labelled DBL allows the user to distinguish between the two beads. Decoding methods and compositions are outlined in more detail in [[U.S.S.N.'s]] U.S.S.N.s 09/189,543, 09/344,526, 60/235,531 and 09/748,706 and WO 99/67641, all of which are expressly incorporated herein by reference.

Please replace the paragraph beginning on page 24, line 3, with the following amended paragraph:

In a preferred embodiment, pyrosequencing techniques are used to decode the array, as is generally described in U.S.S.N.s 60/160,927, 09/513, 362 (US 20030108867), 09/553, 993 and 09/556, 463, hereby expressly incorporated by reference.

Please replace the paragraph beginning on page 29, line 24, with the following amended paragraph:

In a preferred embodiment, decoding of self-assembled random arrays is done on the bases of pH titration. In this embodiment, in addition to target analytes, the beads comprise optical signatures, wherein the optical signatures are generated by the use of pH-responsive dyes (sometimes referred to herein as "ph dyes") such as fluorophores. This embodiment is similar to that outlined in ~~PCT US98/05025~~ WO98/40726 and ~~U.S.S.N. 09/151,877~~ U.S. Patent No. 6,327,410, both of which are expressly incorporated by reference, except that the dyes used in the present invention exhibits changes in fluorescence intensity (or other properties) when the

solution pH is adjusted from below the pKa to above the pKa (or vice versa). In a preferred embodiment, a set of pH dyes are used, each with a different pKa, preferably separated by at least 0.5 pH units. Preferred embodiments utilize a pH dye set of pKa's of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11, and 11.5. Each bead can contain any subset of the pH dyes, and in this way a unique code for the target set is generated. Thus, the decoding of an array is achieved by titrating the array from pH 1 to pH 13, and measuring the fluorescence signal from each bead as a function of solution pH.

Please replace the paragraph beginning on page 42, line 8, with the following amended paragraph:

Accordingly, a preferred embodiment of the methods of the invention is as follows. A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or contacted with a reaction chamber or well comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi. If the dNTP is complementary to the base of the target portion of the target sequence adjacent to the extension primer, the dNTP is added, releasing PPi and generating detectable light, which is detected as generally described in U.S.S.N.~~s 09/151,877 and~~ 09/189,543, U.S. Patent No. 6,327,410, and PCT US98/09163, all of which are hereby incorporated by reference. If the dNTP is not complementary, no detectable signal results. The substrate is then contacted with a second reaction chamber comprising a different dNTP and the additional components of the assay. This process is repeated if the identity of a base at a second detection position is desirable.

Please replace the paragraph beginning on page 49, line 10, with the following amended paragraph:

In addition, a variety of fluid handling techniques find use in the present invention. For example, a variety of microfluidics techniques, such as are generally outlined in U.S.S.N. 09/316,154 (US 20020051971), hereby expressly incorporated by reference. In particular, a system that utilizes a chamber comprising the discrete sites, i.e. wells. The beads are loaded into the chamber using a microfluidic system as generally described in U.S.S.N. 09/316,154 (US 20020051971), 60/252, 227 and ~~PCT/US00/13942~~ WO00/71243.